Short Communication: Seasonal Effects on Development of Bovine Embryos Produced by In Vitro Fertilization in a Hot Environment

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ABSTRACT

The objective of this study was to determine if season affected the production of in vitro-derived bovine embryos from oocytes of cattle in a subtropical environment. Ovaries (~75% beef cattle, including many with *Bos indicus* breeding) were collected from an abattoir. Oocytes were obtained and subjected to in vitro maturation and fertilization. Embryos were then cultured in CR1aa medium. Cleavage rate averaged 72.2 ± 9.7% and was not different between months of collection. In addition, no differences were observed in the percent of oocytes or embryos that became blastocysts on d 8 or 9 after insemination. Least-squares means averaged across months for percent oocytes and cleaved embryos to blastocyst on d 8 were 22.8 ± 7.5% and 31.2 ± 9.4%, respectively. When d 8 blastocysts were classified according to stage of development (nonexpanded, expanded, and hatched), an effect of month was observed that reflected month-to-month variation and not a consistent change associated with season. Taken together, results failed to indicate an effect of season on in vitro production of embryos in a subtropical environment.
(**Key words:** in vitro fertilization, heat stress, seasonality)

Abbreviation key: IVF = in vitro fertilization, **IVP** = in vitro production of embryos.

Heat stress may compromise oocyte quality. Ewes exposed to high temperature 12 d prior to breeding experienced reduced fertilization rates and increased embryonic loss (2). In cattle, heat shock of 41°C during in vitro oocyte maturation decreased the ability of oocytes to become blastocysts following fertilization (3), and heat stress of superovulated cows at the onset of estrus reduced subsequent embryonic development (7). Moreover, effects of heat stress on follicular development (1, 13) could conceivably alter oocyte quality. High environmental temperatures affected the developmental competence of oocytes collected from Holstein cows (8).

Effects of heat stress on oocyte quality could lead to seasonal variation in the success rate of in vitro embryo production systems. Decreased embryo yields in summer have been reported in Louisiana (8) and for Wisconsin (10) in cattle and for The Netherlands in humans (12). The magnitude of seasonal effects is likely to depend on geographical location. Also, the prevailing genotype of cattle providing ovaries may be important in determining the magnitude of seasonal effects because certain breeds of cattle are better able to regulate body temperature during heat stress (4). For the current experiment, we hypothesized that the yield of embryos produced in vitro from ovaries collected in Florida would be lower during the hot months of the year than during the cool months.

In Vitro Embryo Production

Ovaries were obtained from a local abattoir at a travel distance of 1.5 h from the laboratory. Cattle slaughtered at this facility were predominantly beef cattle (~75%; ~10% feedlot cattle, ~65% crossbred range cattle with a variable degree of *Bos indicus* breeding). The other ~25% of the animals slaughtered at this facility were Holstein.

Ovaries were transported to the laboratory at ambient temperature in 0.9% (w/v) NaCl. Procedures for recovery of oocytes, maturation, fertilization, and embryo culture have been reported previously (3, 6). Briefly, ovaries were sliced to obtain cumulus-oocyte complexes that were then matured in groups of 10 in 50-μl microdrops of TCM 199 supplemented with 10% (vol/vol) bovine steer serum, 25 μg of follicle stimulating hormone/ml, 2 μg of estradiol-17β/ml, 150 μg of glutamine/ml, 50 μg of gentamicin/ml and 22 μg of sodium pyruvate/ml for 20 to 24 h at 38.5°C with an atmosphere of 5% CO_2. For fertilization, cumulus-oocyte complexes were washed once in HEPES-TALP (5) and placed in groups of 30 in 600 μl of IVF-TALP (5) and inseminated with Percoll-purified sperm (~1×10^6 sperm cells) pooled

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from three different bulls. Fertilization was allowed to proceed for 8 to 10 h. Embryos/oocytes were denuded of cumulus cells, washed in HEPES-TALP three times and placed in groups of 20 to 30 in 50-μl microdrops of CR1aa medium (9) containing 3 mg of Fraction V bovine serum albumin/ml and 2.5 μg of gentamicin/ml. Embryos were cultured at 38.5°C in 5% CO_2. On d 5 after insemination, 5 μl of heat-inactivated fetal calf serum was added to each microdrop; development was determined on d 7 to 9. Three stages of blastocyst development were recorded: 1) nonexpanded blastocyst (contains an obvious blastocoele, inner cell mass and trophectoderm while being surrounded by a thick zona pellucida), 2) expanded blastocyst (an embryo that has grown considerably with a much larger blastocoelic cavity and a thinned zona pellucida), and 3) hatched blastocyst (a blastocyst that has penetrated the zona pellucida).

Statistics

Data analyzed were from 83 in vitro procedures (**IVP**) conducted by three technicians over a 2-yr period (July 1996 to July 1998). Generally, all in vitro fertilization (**IVF**) procedures conducted during this period were included in the analysis even if cleavage or development rates were very low or zero. The exception was for four runs removed from the analysis due to known causes for failure (i.e., incubator failure, medium contamination). Data collected from each run were percentage of oocytes cleaving, percentage of oocytes and embryos developing to blastocyst, and percentage of blastocysts that were nonexpanded, expanded, or hatched. Data were analyzed by least-squares analysis of variance by PROC GLM procedures of SAS (11). The mathematical model included effects of month of oocyte collection, year of oocyte collection, technician, month × year, and month × technician. All effects were considered fixed. Since percentage data are not normally distributed, data were transformed with an arcsine transformation before analysis. Probability values are presented from the analysis of transformed data but least-squares means ± SEM are presented from analysis of untransformed data.

RESULTS

The percentage of oocytes that cleaved averaged 72.2 ± 9.7% (average across months of least-squares means ± within month SEM); cleavage rate was not different ($P > 0.05$) between months (Figure 1; top panel). The percentage of oocytes and embryos that developed to the blastocyst stage at d 8 after insemination averaged 22.8 ± 7.5% and 31.2 ± 9.4% (average across months of least-squares means ± within month SEM), respectively. There were no significant differences between months (Figure 1; middle and bottom panels). Similar results were seen when development of blastocysts at d 9 after insemination was calculated (results not shown).

The proportion of blastocysts classified as nonexpanded, expanded, and hatched is shown in Figure 2.

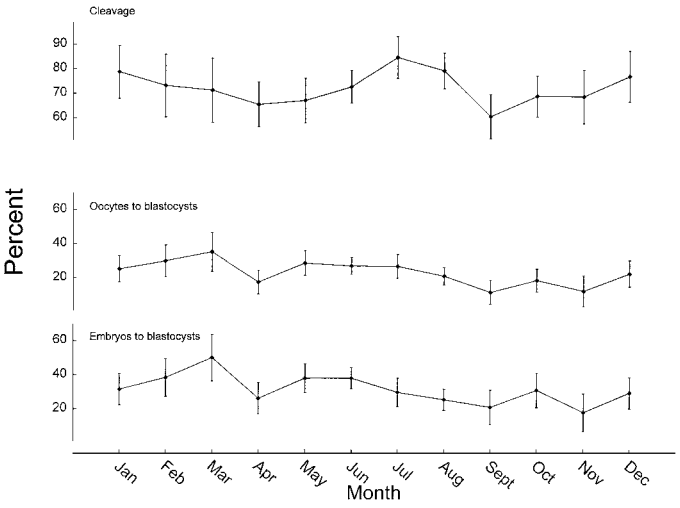

Figure 1. Monthly variation in performance of an in vitro embryo production system in Florida. Results are the percentage of oocytes that cleaved following in vitro maturation and fertilization (top panel), the percentage of oocytes that became blastocysts on d 8 after in vitro fertilization (IVF) (middle panel), and the percentage of cleaved embryos that became blastocysts at d 8 after IVF (bottom panel). Data are least-squares means ± SEM. Data represent 7222 oocytes from 83 IVF procedures (top panel), 5454 oocytes from 71 IVF procedures (middle panel), and 5304 oocytes from 66 IVF procedures (bottom panel). Differences in numbers between the top and middle panels reflect the fact that some embryos were removed for experimental purposes before d 8. Differences in numbers between the middle and bottom panels occurred because no data were available for percentage of embryos to blastocyst for five IVF procedures in which there was complete fertilization failure.

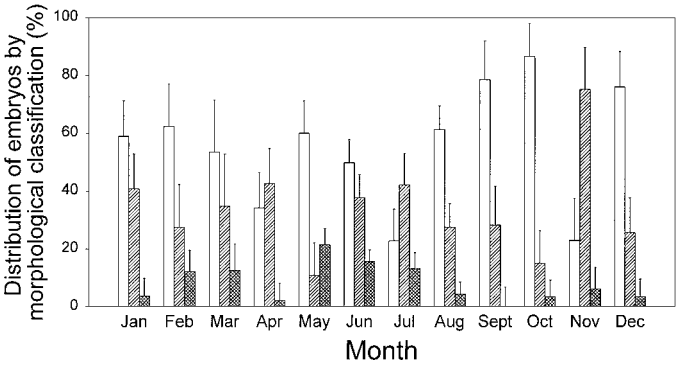

Figure 2. Effect of month on the proportion of d 8 blastocysts that were nonexpanded (open bars), expanded (hatched bars), and hatched (crosshatched bars). Data are least-squares means ± SEM. Data represent 1045 blastocysts from 66 IVF procedures.

At d 8 after insemination, month of the year affected the proportion of blastocysts classified as nonexpanded ($P < 0.005$) and expanded ($P < 0.03$), but not as hatched. Effects of month did not reflect a seasonal pattern in classification of blastocysts but instead reflected considerable variation between months. For example, the proportion of blastocysts classified as expanded was very high in November and lower in May and October when compared to other months. Similarly, the percentage of nonexpanded blastocysts was highest in October and lower in July and November.

Results from the present study indicate no effect of season on cleavage rate or subsequent development of oocytes subjected to IVF from cattle reared in a subtropical environment. This finding is different from a recent report from Wisconsin in which blastocyst yield was reduced during mid to late summer (10). However, those authors used oocytes primarily from Holstein cows; cows in this study included large numbers of crossbred beef cattle with *Bos indicus* genetics. In another report (8), the developmental competence of oocytes was reduced for oocytes collected and cultured in vitro during the hot season in *Bos taurus* (Holstein and crossbred Angus) but not in *Bos indicus* (Brahman) cows. Thermoregulatory ability during periods of heat stress is greater for *Bos indicus* than *Bos taurus* (4).

Another possibility for the difference in results between the present study and earlier reports (8, 10) may lie in differences in the method of oocyte collection. Rutledge et al. (10) collected oocytes from slaughterhouse ovaries by aspiration of follicles (J. J. Rutledge, 1999, personal communication) and Rocha et al. (8) collected oocytes from live cows by using ultrasound-guided follicle aspiration. In contrast, ovaries in the present study were obtained by slicing the ovaries. Perhaps the population of follicles harvested for oocyte collection varies between methods. With slicing, more oocytes are recovered than for aspiration. Harvested follicles tend to be smaller on average and these may yield oocytes that are less heat-sensitive than oocytes from follicles collected by aspiration.

The present results indicate that it is possible to produce embryos in vitro in a subtropical environment without adverse effects of season associated with warm temperatures. The failure to find an effect of season could reflect greater resistance of beef cattle and particularly *Bos indicus* cattle to warm environments. Alternatively, it is possible that heat stress does not cause permanent damage to the functional competence of the population of oocytes recovered using the slicing method.

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